Astrocyte-enriched feeder layers from cryopreserved cells support differentiation of spontaneously active networks of human iPSC-derived neurons.

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Public Summary:
BACKGROUND: Human induced pluripotent stem cell (hiPSC)-derived neuronal cultures are a useful tool for studying the mechanisms of neurological disorders and developing novel therapeutics. While plating hiPSC-derived neuronal progenitors onto glial feeder layers prepared from rodent cortex has been reported to promote functional differentiation of neuronal networks, this has not been examined in detail. NEW METHOD: Here we describe a method of using cryopreserved cells from primary cultures for generation of mouse astrocyte-enriched, neuron-free feeder layers that grow from 10% to 100% confluence in 1 week. RESULTS: Electrophysiological analysis demonstrated that compared to biochemical substrates alone, astrocyte-enriched feeder layers support more rapid differentiation of hiPSC-derived progenitors into excitable neurons that form spontaneously active networks in culture. There was a positive correlation between the degree of astroglial confluence at the time of progenitor plating and the average frequency of postsynaptic currents 3 weeks after plating. One disadvantage to plating on 100% confluent feeder layers was a high incidence of the astroglial layer with the overlying neurons detaching from the coverslips during transfer to the recording chamber. COMPARISON WITH EXISTING METHOD(S): Prevailing methods using primary glial feeder layers can result in possible contamination with rodent neurons and an unpredictable rate of growth. We provide a reliable method of generating mouse astroglial feeder layers from cryopreserved primary cultures to support differentiation of hiPSC-derived neurons. CONCLUSIONS: The ability to make astrocyte-enriched feeder layers of defined confluence from cryopreserved primary cultures will facilitate the use of human stem cell derived neuronal cultures for disease modeling.

Scientific Abstract:
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